



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 :

A61K 37/26, 9/16, 9/50

A1

(11) International Publication Number:

WO 92/06704

(43) International Publication Date:

30 April 1992 (30.04.92)

(21) International Application Number: PCT/US91/07475

(22) International Filing Date: 10 October 1991 (10.10.91)

(30) Priority data:

595,468

10 October 1990 (10.10.90) US

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(81) Designated States: AT (European patent), AU, BE (European patent), BR, CA, CH (European patent), DE (European patent), DK, DK (European patent), ES (European patent), FI, FR (European patent), GB (European patent), GR (European patent), HU, IT (European patent), JP, KR, LU (European patent), NL (European patent), NO, SE (European patent).

Published

With international search report.

(54) Title: METHOD OF TREATING OR PREVENTING TYPE I DIABETES BY ORAL ADMINISTRATION OF INSULIN

## (57) Abstract

Disclosed herein are methods for treating or preventing a disease in mammals having the characteristics of Type I diabetes comprising administering insulin or disease suppressive fragments of insulin or analogs thereof in oral or aerosol dosage forms to said mammals. Also disclosed herein are pharmaceutical formulation or dosage forms for use in the methods.

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METHOD OF TREATING OR PREVENTING TYPE 1DIABETES BY ORAL ADMINISTRATION OF INSULINFIELD OF THE INVENTION

This invention relates to methods and compositions  
15 for treating or preventing Type 1 diabetes mellitus. The  
methods of the invention do not require parenteral administra-  
tion of therapeutic agents. More particularly, but not by way  
of limitation, the present invention is directed to agents and  
methods for treating or preventing autoimmune diseases having  
20 the characteristics of Type 1 diabetes mellitus.

BACKGROUND OF THE INVENTION

Diabetes mellitus is characterized in two broad  
groups based on clinical manifestations, namely, the non-  
25 insulin-dependent or maturity onset form, also known as Type 2;  
and the insulin-dependent or juvenile onset form, also known as  
Type 1. Clinically, the majority of Type 2, maturity onset  
diabetics are obese, with manifestations of clinical symptoms  
of the disease usually appearing at an age over 40. In con-  
30 trast, Type 1, juvenile onset patients are not over-weight

relative to their age and height, with rapid onset of the disease at an early age, often before 30, although Type 1 diabetes can occur at any age.

Diabetes mellitus is a metabolic disorder in humans with a prevalence of approximately one percent in the general population, with one-fourth of these being the Type 1, insulin-dependant category (Foster, D.W., Harrison's Principles of Internal Medicine, Chap. 114, pp. 661-678, 10th Ed., McGraw-Hill, New York). The disease manifests itself as a series of hormone-induced metabolic abnormalities which eventually lead to serious, long-term and debilitating complications involving several organ systems including the eyes, kidneys, nerves, and blood vessels. Pathologically, the disease is characterized by lesions of the basement membranes, demonstrable under electron microscopy.

Type 1 diabetics characteristically show very low or immeasurable plasma insulin with elevated glucagon. Regardless of what the exact etiology is, most Type 1 patients have circulating antibodies directed against their own pancreatic cells including antibodies to insulin, to islet of Langerhans cell cytoplasm and to the enzyme glutamic acid decarboxylase. An immune response specifically directed against beta cells (insulin producing cells) leads to Type 1 diabetes. This specificity is supported by the above clinical picture, since beta cells secrete insulin while alpha cells secrete glucagon.

Current therapeutic regimens for Type 1 diabetes include modifications to the diet in order to minimize hyper-

glycemia resulting from the lack of natural insulin, which in turn, is the result of damaged beta cells. Diet is also modified with regard to insulin administration to counter the hypoglycemic effects of the hormone. Whatever the form of treatment, parenteral administration of insulin is required for all Type 1 diabetics, hence the term "insulin-dependent" diabetes.

Conventional insulin therapy is limited to the parenteral (i.e. subcutaneous) administration of insulin. Oral administration of insulin has not been possible because the insulin molecule cannot pass through the digestive tract in a sufficiently intact form to provide its therapeutic benefit. There has been an ongoing search by those skilled in the art for an alternative method to eliminate or reduce the need for insulin because of the numerous problems associated with subcutaneous administration of the drug. Among the methods that have been investigated are implantable insulin pumps and pancreatic islet cell transplantation.

Because Type 1 diabetes usually manifests itself in adolescents and because the subcutaneous delivery of insulin requires strict, self-regimentation, compliance is often a serious problem. In addition, the act of the parenteral administration itself can be very traumatic to juveniles. For the clinician, it is difficult to precisely regulate the amounts of insulin needed at any given time of the patient's day. Furthermore, it is all but impossible to regulate blood glucose levels in diabetic patients with parenteral insulin to

the extent to which blood glucose is regulated in normal individuals.

Thus, in the early stages of treatment of Type 1 diabetes, patients often become either hyperglycemia or hypoglycemic because the exact timing of the insulin injections and levels of insulin needed are not known. As treatment progresses the clinician and, more importantly, the patient adjusts to the daily routine, but there is always the risk of ketoacidosis or hypoglycemia.

10 In addition, some patients produce antibodies to the injected insulin even though most patients are now treated with human insulin produced by recombinant technology. This can lead to the need for higher doses of insulin. To date, there has been no successful oral dosage form containing  
15 insulin.

The art has long sought a method for preventing and/or treating Type 1 diabetes which does not involve parenteral administration of insulin. Various attempts at alternate delivery methods for insulin have failed or have not progressed  
20 to a practical level. For example, oral insulin, transcutaneous insulin delivery, and nasal insulin have not become clinically utilized. Oral insulin does not affect blood glucose levels. Because it has been necessary to inject lipids into mammals receiving nasal insulin in order to get the insulin  
25 across the nasal mucosa, this route of insulin administration has been limited.

Oral dosage forms for treatment of Type 2 diabetes are available, (e.g. oral sulfonylureas). These orally administered agents do not comprise insulin but rather stimulate the pancreas to produce insulin, and are only effective for treatment of certain forms of Type 2 diabetes. As for Type 1 diabetes, oral agents such as sulfonylureas are ineffective due to markedly diminished or damaged beta-cell mass (Foster, D.W., Harrison's Principles of Internal Medicine, Chap. 114, p. 668, 10th Ed., McGraw-Hill, New York).

Type 1 diabetes is considered to be a disease of autoimmune etiology (Eisenbarth, G.S., New Engl. J. Med. 314: 1360-1368, 1986). Various animal models are available for the study of Type 1 diabetes as an autoimmune disorder. These include the BB mouse (Nakbookda, A.F., et al., Diabetologic 14: 199-207, 1978) and the NOD (non-obese diabetic) mouse in which diabetes develops spontaneously (Prochazka et al. Science 237:286, 1987). Islet-cell specific, CD4 and CD8 T-lymphocytes have been implicated as causative agents for damage to beta cells, demonstrated by decreased incidence of Type 1 diabetes in NOD mice (J. Exp. Med. 166:823, 1987).

Other therapies are being developed for the treatment of autoimmune diseases in general.

Weiner et al., U.S. Patent Application Ser. No. 460,852 filed February 21, 1990, (the national stage of PCT Application No. PCT/US88/02139, filed June 24, 1988), which is a continuation-in-part application of U.S. Patent Application Ser. No. 065,734 filed June 24, 1987, discloses the treatment

of autoimmune diseases by oral administration of autoantigens.

Weiner et al., U.S. Patent Application Ser. No. 454,486 filed December 20, 1989, discloses the aerosol administration of autoantigens, disease-suppressive fragments of said autoantigens and analogs thereof as an effective method for treating T-cell mediated autoimmune diseases.

Weiner et al., U.S. Patent Application Ser. No. 487,732, filed March 2, 1990, discloses synergists (enhancers) for use with oral administration of autoantigens, disease suppressive fragments and analogs thereof as effective treatments for T-cell mediated autoimmune diseases.

Weiner et al., U.S. Patent Application Ser. No. 551,632 filed July 10, 1990, as a Rule 62 continuation-in-part application of U.S. Patent Application Serial No. 379,778, filed July 14, 1989 (now abandoned), discloses methods of preventing or treating uveoretinitis in mammals by oral administration of purified S antigen.

Nagler-Anderson, et al., (Proc. Natl. Acad. Sci (USA) 83: 7443-7446, 1986), describe the oral administration of collagen to suppress collagen-induced arthritis in a mouse model.

However, none of the above-mentioned for treatments for autoimmune diseases can be used treating Type 1 diabetes because the antigens involved in eliciting and maintaining the Type 1 disease have not been identified.



It is, therefore, an object of the present invention to provide agents and methods for treating and preventing autoimmune diseases in mammals having the characteristics of Type 1 diabetes. Prevention of Type 1 diabetes preferably  
5 involves treatment prior to hyperglycemia.

Another object of the present invention is to provide compositions and pharmaceutical formulations useful for treating mammals suffering from autoimmune diseases having the characteristics of Type 1 diabetes.

10 A still further object of the invention is to provide compositions and pharmaceutical formulations useful for oral administration to mammals for the purpose of preventing or attenuating the manifestations (i.e. clinical symptoms) of autoimmune diseases having the characteristics of Type 1  
15 diabetes. Prevention of Type 1 diabetes includes treatment prior to hyperglycemia.

These and other objects of the present invention will be apparent to those of ordinary skill in the art in light of the following.

20

#### SUMMARY OF THE INVENTION

It has unexpectedly been discovered that oral administration of insulin is an effective treatment for eliminating or reducing the need for insulin in Type 1 diabetics. Oral  
25 insulin can prevent or ameliorate beta cell destruction and thereby decrease or eliminate traditional parenteral insulin therapy.

Orally administerable pharmaceutical formulations containing insulin are prepared and administered to mammals who have manifested symptoms of Type 1 diabetes and/or diagnosed as having Type 1 diabetes. Additionally, subjects who are at risk  
5 for developing Type 1 diabetes (i.e. have demonstrated a predisposition to developing Type 1 diabetes through suitable means, such as genetic studies and analysis) are treated with similar oral preparations of insulin.

Pharmaceutical formulations for oral or enteral administration to treat Type 1 diabetes are prepared from commercially  
10 available insulin and a pharmaceutically acceptable carrier suitable for oral ingestion. The quantity of insulin in each dose may be between 1 mg and 1000 mg. However the total dose required for treatment varies according to the individual.  
15 Generally, the total quantity of insulin required in practicing the present invention is a much larger dose than is the dosage that is administered parenterally to protect an individual afflicted with Type 1 diabetes against ketoacidosis.

Additionally, an aerosol delivery system can be made  
20 with similar dosages of insulin as above with a pharmaceutically suitable carrier or diluent. These and other improvements will be described in the following descriptions, drawings and the appended claims.

25 BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a graph showing the effects of orally administered porcine insulin on diabetes in NOD mice.

Figure 2 is a graph showing the effects of oral porcine insulin on serum glucose concentrations post-administration of oral insulin in NOD mice.

Figure 3 is a graph showing the effect of oral administration of pancreatic extracts given at weekly intervals on the development of diabetes in NOD mice.

#### DETAILED DESCRIPTION OF THE INVENTION

All patent applications, patents and literature references referred to herein are hereby incorporated by reference in their entirety.

The present invention addresses the need for an alternate to existing methods for the treatment of Type 1 diabetes from an autoimmune model and in particular at treatment for the prevention of diabetes. Because Type 1 patients are predominantly adolescents, it is believed that long term insulin therapy will no longer be required due to preservation of undamaged beta cell function if treatment is initiated at the onset of the disorder using the compositions and methods of the present invention. In addition, if the methods of the present invention are initiated at a time when some beta cell function still exists, parenteral insulin therapy can be reduced. Thus, the present invention provides means whereby long term insulin therapy would be reduced or no longer be required.

It has now been unexpectedly discovered that oral and/or aerosol administration of insulin (or disease-suppres-

sive fragments of insulin or analogs thereof) is effective for the treatment and prevention of Type 1 diabetes. This is a radical departure from traditional (parenteral) insulin therapy in that insulin is administered in order to damp down or shut  
5 off the hosts autoimmune response and not for its endocrinologic (metabolic) effect. Oral administration of insulin has not proved to be effective in treating any form of diabetes because proteolytic enzymes present in the stomach and digestive system degrade the polypeptide before it can reach  
10 the bloodstream. In addition, intranasal administration of insulin has also not previously been shown to be effective in treating any form of diabetes.

Without wishing to be bound by theory of operation it is believed that the oral or aerosol administration of insulin  
15 pursuant to the present invention affects the immunological pathogenesis of Type 1 diabetes through the elicitation of suppressor T-cells.

In the following discussions the following terms shall have the meaning ascribed to them below.

20 "Treatment" shall mean the treatment of active disease in patients with some level of undamaged, insulin-producing beta cells as well as prophylactic administration for use in patients having a high risk for developing the disease.

"Oral administration" shall mean both oral administration and enteral administration (direct incubation into the  
25 stomach).

"Individuals at risk" for Type 1 diabetes shall mean

- a) individuals having a blood relative with Type 1 diabetes; b) autoantibody-positive individuals without overt Type 1 diabetes. These auto antibodies include cytoplasmic islet cell  
5 autoantibodies, insulin antibodies and glutamic acid decarboxylase autoantibodies; c) individuals with Histocompatibility (HLA) type DR3 or DR4DQW8; d) individuals with glucose abnormalities such as a loss of first phase insulin secretion on glucose tolerance tests.

- 10 "Mammal" shall mean any organism having an immune system and therefore susceptible to Type 1 diabetes.

"Active disease", shall mean autoimmune destruction of islet beta cells.

- "Aerosol" refers to finely divided solid or liquid  
15 particles that may be created using a pressurized system such as a nebulizer. The liquid or solid source material contains insulin and/or disease suppressive fragments of insulin and analogs thereof as defined herein.

- "Disease suppressive fragments" of insulin includes  
20 any peptide or polypeptide containing partial amino acid sequences or moieties of insulin and possessing the ability to treat or prevent a disease having the characteristics of Type 1 diabetes. Such fragments need not possess the autoantigenic or endocrinologic (metabolic) properties of the entire insulin  
25 molecule. *f*

"Analog" of insulin or disease suppressive fragments thereof refers to compounds that are structurally related to

insulin or disease suppressive fragments thereof which possess the same biologic activity, i.e., the ability to suppress or prevent disease symptoms of Type 1 diabetes, upon oral or aerosol administration. By way of non-limiting example, the term includes peptides having amino acid sequences which differ from the amino acid sequence of insulin or disease suppressive fragments thereof by one or more amino acid residues while still retaining the disease suppressive activity of insulin or its ability to prevent or alleviate the symptoms of Type 1 diabetes. These analogs do not necessarily need to possess the endocrinologic effects of insulin.

In accordance with the present invention, experiments were performed in which NOD (non-obese diabetic) mice, which develop diabetes spontaneously between 11 and 52 weeks of age, had a lower incidence of diabetes at all doses of orally administered insulin in a dose-dependent manner. In all of the animals which received the highest dose of oral insulin (1 mg), 100% of the treated animals failed to develop diabetes.

Although it is fairly easy to control the symptoms of Type 1 diabetes with parenteral insulin, it is difficult to normalize a patient's blood sugar throughout 24 hours utilizing traditional insulin therapy given as 1 or 2 injections a day. Thus, the present invention provides a method for treating or preventing a disease having the characteristics of Type 1 diabetes in a mammal comprising administering to a mammal, suffering from or at risk for Type 1 diabetes, an effective

amount of an oral insulin dosage form to treat or prevent the symptoms of diabetes.

It should be noted that the methods of the present invention will not eliminate the need for parenteral insulin therapy in patients with damaged beta cells who do not produce enough insulin to regulate their blood sugar. However, using the methods and compositions of the present invention, newly diagnosed Type 1 diabetic patients or those at risk for the disease (as defined above), who have substantially intact (undamaged) beta cells, will not go on to develop Type 1 diabetes and parenteral insulin administration may be eliminated. In addition, it is believed that the methods and compositions of the present invention will reduce the amount of insulin needed by those individuals having some beta cell function (who are able to produce some level of insulin) by halting progression of the disease.

Insulin for use in the present invention can be obtained from numerous commercial sources such as Novo Laboratories (Danbury, CT), Nordisk-USA (Rockville, MD) and Eli Lilly and Co. (Indianapolis, IN). Porcine-derived insulin, human semi-synthetic insulin (Nordisk-USA) and cloned recombinant insulin (Eli Lilly) can be used when practicing the method of the present invention.

Disease suppressive fragments and analogs of insulin for use in the present invention can be synthesized using well known solid phase synthesis techniques (Merrifield, R.B. Fed. Proc. Am. Soc. Ex. Biol. 21: 412, 1962 and J. Am. Chem. Soc.

85: 2149, 1963; Mitchel, A.R. et al., J. Am. Chem. Soc. 98: 7357, 1976; Tam, J. et al., J. Am. Chem. Soc. 105: 6442, 1983).

Analogues can be constructed by identifying an equivalent amino acid sequence and using the peptide synthesis techniques

5 disclosed above.

Analogues can be provided using the known amino acid sequence of insulin as disclosed in Atlas of Protein Sequence and Structure, Natl. Biochem. Res. Foundation, vol. 5, pgs. 209-211.

10 Disease-suppressive analogues and fragments can also be obtained using recombinant DNA techniques well-known in the art.

Disease suppressive fragments of insulin and analogues thereof can be identified using routine experimentation using  
15 suitable in vivo systems such as those of Examples 1-5 below.

Pursuant to the present invention, insulin or disease suppressive fragments or analogues thereof are introduced into a mammal suffering from or at risk for a disease having the characteristics of Type 1 diabetes, orally or enterally, in an  
20 amount of between about 2 mg per kg body weight of said mammal and about 10 mg per kg body weight of said mammal per day, and may be administered in a single dose form or multiple dose forms. Preferably, the insulin is administered in an amount  
between about 2.5 mg and about 5.0 mg per kg body weight of  
25 said mammal per day. The exact amount to be administered will vary depending on the severity and stage of a patient's disease and the physical condition of the patient.



The present invention also is directed to oral dosage forms and pharmaceutical formulations for administration to mammals suffering from or at risk for diseases having the characteristics of Type 1 diabetes. It will be understood that  
5 any statistically significant attenuation in the disease symptoms of Type 1 diabetes pursuant to the treatment of the present invention is within the scope of the invention.

Each oral formulation according to the present invention may additionally comprise inert constituents including  
10 pharmaceutically acceptable carriers, diluents, fillers, solubilizing or emulsifying agents and salts as is well-known in the art. For example, tablets may be formulated in accordance with conventional procedures employing solid carriers well-known in the art. Capsules employed in the present  
15 invention may be made from any pharmaceutically acceptable material such as gelatin or cellulose derivatives. Sustained release oral delivery systems and/or enteric coatings for orally administered dosage forms are also contemplated such as those described in U.S. Patent No. 4,704,295 issued November 3,  
20 1987, U.S. Patent No. 4,556,552 issued December 3, 1985, U.S. Patent No. 4,309,404 issued January 5, 1982 and U.S. Patent No. 4,309,406 issued January 5, 1982.

Examples of solid carriers include bentonite, silica and other commonly used carriers. Further non-limiting exam-  
25 ples of carriers and diluents which may be used in the formulations of the present invention include saline and any physio-

logically buffered saline solution such as phosphate buffered saline (PBS) and water.

It will be appreciated that the unit content of active ingredient or ingredients contained in an individual  
5 dose of each dosage form need not in itself constitute an effective amount since the necessary effective amount can be reached by administration of a plurality of dosage units.

The preferred route of administration of the dosage forms of the present invention is orally or enterally. Preferred oral or enteral pharmaceutical formulations or dosage  
10 forms may comprise for example, between about 1 mg and about 1000 mg of insulin.

In an alternative preferred embodiment of the present invention the pharmaceutical formulations or dosage forms of  
15 the present invention can also be administered to mammals suffering from diseases having the characteristics of Type 1 diabetes in aerosol form. It is expected that lower amounts of insulin, disease suppressive fragments or analogs thereof will be required using aerosol administration for treating or  
20 preventing Type 1 diabetes as has been found when treating experimental allergic encephalomyelitis (EAE) with myelin basic protein (MBP) and adjuvant arthritis with collagen as disclosed in co-pending U.S. Patent Application Ser. No. 454,486 filed  
December 20, 1989. The amounts of insulin or disease suppressive fragments or analogs thereof which may be administered in  
25 an aerosol dosage form would be between about 0.1 mg and 10 mg per kg body weight of a mammal per day and may be administered

in single dosage form or multiple dosage forms. The exact amount to be administered will vary depending on the state and severity of a patient's disease and the physical condition of the patient.

5           The aerosol pharmaceutical formulations of the present invention may include, as optional ingredients, pharmaceutically acceptable carriers, diluents, solubilizing or emulsifying agents, and salts of the type that are well-known in the art. Examples of such substances include normal saline  
10 solutions, such as physiologically buffered saline solutions, and water.

          The route of administration of insulin or disease suppressive fragments or analogs thereof according to this alternate embodiment of the present invention is in an aerosol  
15 or inhaled form. The insulin and related compounds of the present invention can be administered as a dry powder or in an aqueous solution. Preferred aerosol pharmaceutical formulations may comprise for example, a physiologically-acceptable buffered saline solution containing between about 1 mg and  
20 about 1000 mg of insulin, disease suppressive fragments or analogs thereof.

          Dry aerosol in the form of finely divided solid particles of insulin, disease suppressive fragments or analogs thereof that are not dissolved or suspended in a liquid are  
25 also useful in the practice of the present invention. The insulin may be in the form of dusting powders and comprise finely divided particles having an average particle size of

between about 1 and 5 microns, preferably between 2 and 3 microns. Finely divided particles may be prepared by pulverization and screen filtration using techniques well known in the art. The particles may be administered by inhaling a  
5 predetermined quantity of the finely divided material, which can be in the form of a powder.

Specific non-limiting examples of the carriers and/or diluents that are useful in the aerosol pharmaceutical formulations of the present invention include water and physiological-  
10 ly-acceptable buffered saline solutions such as phosphate buffered saline solutions pH 7.0-8.0.

The pharmaceutical formulations of the present invention may be administered in the form of an aerosol spray using for example, a nebulizer such as those described in U.S. Patent  
15 Nos. 4,624,251 issued November 25, 1986; 3,703,173 issued November 21, 1972; 3,561,444 issued February 9, 1971 and 4,635,627 issued January 13, 1971. The aerosol material is inhaled by the subject to be treated.

Other systems of aerosol delivery, such as the pressurized metered dose inhaler (MDI) and the dry powder inhaler  
20 as disclosed in Newman, S.P. in Aerosols and the Lung, Clarke, S.W. and Davia, D. eds. pp. 197-224, Butterworths, London, England, 1984, can be used when practicing the present invention.

25 Aerosol delivery system of the type disclosed herein are available from numerous commercial sources including Fisons

Corporation (Bedford, MA), Schering Corp. (Kenilworth, NJ) and American Pharmoseal Co., (Valencia, CA).

It is expected that the methods of the present invention are particularly well-suited for use in pediatric or  
5 adolescent patients developing Type 1 diabetes or the first year of onset of hyperglycemia in whom the secondary effects of the disease (such as vascular damage, kidney damage and diabetic retinopathy) have not been totally manifested. These are also the patients in whom the traumatic effects of daily  
10 insulin injections are usually most severely felt.

As shown in the examples presented below, oral administration of insulin to NOD mice reduced the numbers of these mice which subsequently went on to develop diabetes at all dosages tested. In addition, at the highest dose used (1  
15 mg), none of the mice went on to develop diabetes. In addition, as shown in Example 2, the effects of oral insulin were not due to a reduction in the serum glucose concentrations in these animals and was thus not a metabolic effect. In addition, as shown in Example 3 below, feeding NOD mice pancreatic  
20 extract also led to a diminution in the number of animals which developed diabetes although to a lesser extent than those that received oral insulin.

Preliminary experiments have shown that NOD mice fed 1 mg of insulin had fewer inflammatory immune system cells  
25 surrounding the pancreas (a condition known as insulinitis) than PBS-fed NOD mice (data not shown). Therefore, oral administration of insulin to NOD mice appeared to be affecting the

infiltration of immune system cells into the pancreas and thus halting disease progression by preserving beta cell function.

The present invention is described further below in working examples which are intended to illustrate the present invention without limiting its scope.

EXAMPLE 1:      EFFECT OF FEEDING PORCINE-INSULIN  
                         ON SPONTANEOUS DIABETES IN NOD MICE

10                    NOD (non-obese diabetic mice) were obtained from Taconic Laboratories (Germantown, MA) at 4 weeks of age. There were a total of 30 animals in each of the groups mentioned below at the beginning of the experiment. At 4 1/2 weeks of age, animals were orally administered Porcine insulin (Novo  
15 Laboratories, Danbury, CT). Each animal received two treatments orally twice a week until 9 weeks of age. The mice were fed either saline (control) or 10 micrograms, 100 micrograms, or 1 milligram of Porcine insulin. The animals were fed (gavaged) with an 18 gauge ball-point needle (Popper and Sons,  
20 Inc., New Hyde Park, NY). Beginning at 10 weeks the animals were then fed once a week for a total of 33 weeks. Beginning at 12 weeks the urine was tested weekly for the presence of glucose using Glucosuria test tape (Eli Lilly, Indianapolis, IN). If an animal tested 3+ or higher on the glucose urine  
25 test, a serum sample was taken and blood glucose measured using a glucose analyzer (Beckman). If the blood glucose was 220 or higher, the animals were classified as diabetic and then sacrificed.

As shown in Figure 1, no diabetes developed in any of the animals fed 1 mg insulin whereas diabetes developed in the control group and in the 10 microgram and 100 microgram groups in a dose response fashion. The p value comparing the 1 mg fed to the control is  $p < 0.008$  whereas in the 100 microgram fed,  $p < 0.09$ .

The normal blood insulin level in an animal is between 120 to 170 mg/decaliter or mg/dl. The blood sugars in the animals that were fed PBS that developed diabetes were: 575, 485, 459, 500, 375, 400, 362, 395, 480 mg/dl. The blood sugar in the animals that were fed 10 micrograms of insulin which developed diabetes were: 330, 420, 315, 459, 520, 487 mg/dl. Blood sugars in animals fed 100 micrograms of insulin that became diabetic were: 290, 600, 450, 500 mg/dl. Animals that were fed 1 mg of insulin were also tested in terms of blood sugar. None of the animals developed diabetes as measured by urine testing but the blood sugar was tested as a confirmation. Blood sugar on 10 representative animals from the group fed 1 mg insulin were: 174, 128, 125, 125, 145, 123, 136, 155, 115, 130 mg/dl.

**EXAMPLE 2: GLUCOSE POST ORAL INSULIN IN FASTING 7 WEEK NOD MICE**

In order to determine whether any endocrinologic (i.e. direct lowering of blood sugar) effects occurred following the feeding of insulin, 7 week old NOD mice (5 mice per group) were fed 1 mg of porcine insulin or 1 mg of myelin basic

protein (obtained from Pel Freez, Rogers, AR) as a control protein. Blood glucose was then measured over the next 24 hour period. As a positive control, a separate group of animals were injected subcutaneously with 20 micrograms of Porcine  
5 insulin. All animals were kept in the fasting state.

As shown in Figure 2, animals given subcutaneous insulin had an immediate drop in the glucose level with a decrease of approximately 80 mg % that persisted over a 4 hour period and then slowly returned to normal. Animals which  
10 received oral insulin or oral myelin basic protein had no drop in the blood glucose; in fact there was an increase in blood glucose most probably related to the protein load. Over the next 24 hours there was a gradual decrease in blood glucose in all groups of animals since the animals were in a fasting  
15 state. This was seen both in animals fed insulin and animals fed myelin basic protein.

These results demonstrate that there were no endocrinologic (metabolic) effects from feeding oral insulin. Given the published data in terms of generating immunologic  
20 tolerance and immunologic effects following the oral administration of proteins these results strongly suggest that oral administration of insulin affects the immunologic pathogenesis of diabetes in the NOD mice.



EXAMPLE 3:        EFFECT OF ORAL ADMINISTRATION OF  
                      PANCREATIC EXTRACT GIVEN AT WEEKLY INTERVALS

An identical experiment to Example 1 was performed in  
5 which NOD mice (14-17 per group) were fed one homogenized  
pancreas derived from Lewis rats in 0.5 mls PBS. As shown in  
Figure 3, there was a decrease in the development of diabetes  
in extract-fed animals as compared to controls. Nonetheless,  
the effect is not as dramatic and complete as that seen in the  
10 animals fed insulin. These results show some effect of feeding  
pancreatic tissue and highlight the potency of the insulin  
experiments described above.

15 EXAMPLE 4:        EFFECT OF AEROSOL ADMINISTRATION OF INSULIN ON  
                      DIABETES IN NOD MICE

Insulin (0 to 1 mg as in Examples 1 and 2) will be  
administered to NOD mice as described above in Examples 1 and 2  
except in aerosol form by nebulizer.

20 It is expected that aerosolized insulin will be  
effective in preventing the manifestation of symptoms (i.e.  
hyperglycemia) of Type 1 diabetes in the treated mice.

25 EXAMPLE 5:        TREATMENT OF INITIAL STAGES OF DIABETES IN NOD  
                      MICE

Insulin (0 to 1 mg as in Examples 1 and 2) will be  
administered to NOD mice which are suffering from hyperglycemia  
in an oral and aerosol form. The treatments will begin by  
weeks 12-20 of age, by which the serum glucose levels are at

hyperglycemic concentrations in a certain percentage of animals prior to onset of diabetes.

It is expected that both oral and aerosol administration of insulin will lead to reduction in serum glucose concentrations to near normal levels and preservation of beta cell function.

What is claimed is:

1           1. A method for treating a mammal at risk for a  
2 disease having the characteristics of Type 1 diabetes compris-  
3 ing orally or enterally administering to said mammal in need of  
4 such treatment an effective amount of an agent selected from  
5 the group consisting of insulin, disease suppressive fragments  
6 of insulin and analogs thereof sufficient to prevent said  
7 disease.

1           2. A pharmaceutical formulation for administration  
2 to a mammal at risk for a disease having the characteristics of  
3 Type 1 diabetes comprising an oral dosage form containing an  
4 effective amount for preventing the onset of Type 1 diabetes of  
5 an agent selected from the group consisting of insulin, disease  
6 suppressive fragments of insulin and analogs thereof.

1           3. The pharmaceutical formulation of claim 2  
2 wherein said oral dosage form is a solid dosage form selected  
3 from the group consisting of a tablet, a capsule and a caplet.

1           4. The pharmaceutical formulation of claim 2  
2 wherein the oral dosage form comprises an aqueous suspension  
3 solution of insulin.

1           5. The pharmaceutical formulation of claim 2  
2 comprising a pharmaceutically acceptable carrier or diluent.

1           6. A method for treating a mammal suffering from a  
2 disease having the characteristics of Type 1 diabetes compris-  
3 ing administering to said mammal in need of such treatment an  
4 effective amount for treating said disease of an agent selected  
5 from the group consisting of insulin, disease suppressive  
6 fragments of insulin and analogs thereof in an aerosol form.

1           7. A pharmaceutical formulation for administration  
2 to a mammal suffering from a disease having the characteristics  
3 of Type 1 diabetes comprising an aerosol dosage form containing  
4 an effective amount for treating said disease of an agent  
5 selected from the group consisting of insulin, disease suppres-  
6 sive fragments of insulin and analogs thereof.

1           8. The pharmaceutical formulation of claim 7  
2 comprising a pharmaceutically acceptable carrier or diluent.

1           9. A method for treating a mammal suffering from a  
2 disease having the characteristics of Type 1 diabetes compris-  
3 ing orally or enterally administering to said mammal in need of  
4 such treatment an aerosol dosage form containing an effective  
5 amount sufficient to treat Type 1 diabetes of an agent selected  
6 from the group consisting of insulin, disease suppressive  
7 fragments of insulin and analogs thereof.

1           10. A pharmaceutical formulation for administration  
2 to a mammal suffering from a disease having the characteristics

3 of Type 1 diabetes comprising an aerosol dosage form containing  
4 an effective amount for treating Type 1 diabetes of an agent  
5 selected from the group consisting of insulin, disease suppres-  
6 sive fragments of insulin and analogs thereof.

1 11. The pharmaceutical formulation of claim 10  
2 comprising a pharmaceutically acceptable carrier or diluent.

1 12. A method for treating a mammal at risk for a  
2 disease having the characteristics of Type 1 diabetes compris-  
3 ing administering to said mammal in need of such treatment an  
4 effective amount for preventing said disease of an agent  
5 selected from the group consisting of insulin, disease suppres-  
6 sive fragments of insulin and analogs thereof in an aerosol  
7 form.

1 13. A pharmaceutical formulation for administration  
2 to a mammal at risk for a disease having the characteristics of  
3 Type 1 diabetes comprising an effective amount for preventing  
4 said disease of an agent selected from the group consisting of  
5 insulin, disease suppressive fragments of insulin and analogs  
6 thereof in an aerosol form.

1 14. The pharmaceutical formulation of claim 13  
2 comprising a pharmaceutically acceptable carrier or diluent.

1           15. A method for preventing the onset of Type 1  
2 diabetes in a mammal which comprises the steps of:  
3           testing said mammal to confirm that said mammal  
4 is at risk for developing Type 1 diabetes, and  
5           orally administering an effective amount for  
6 preventing the onset of Type 1 diabetes to said mammal of an  
7 agent selected from the group consisting of insulin, disease  
8 suppressure fragments of insulin and analogs thereof.

1/3

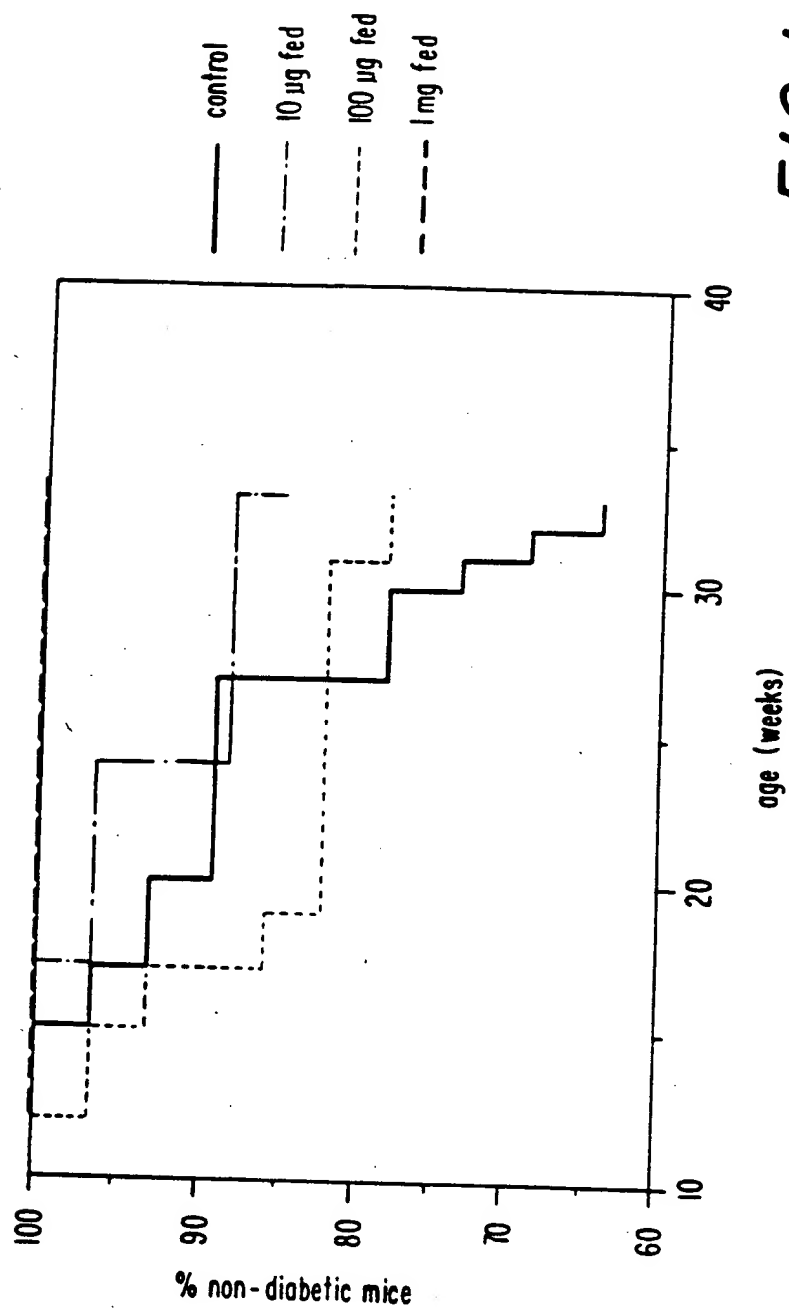


FIG. 1

SUBSTITUTE SHEET





2/3

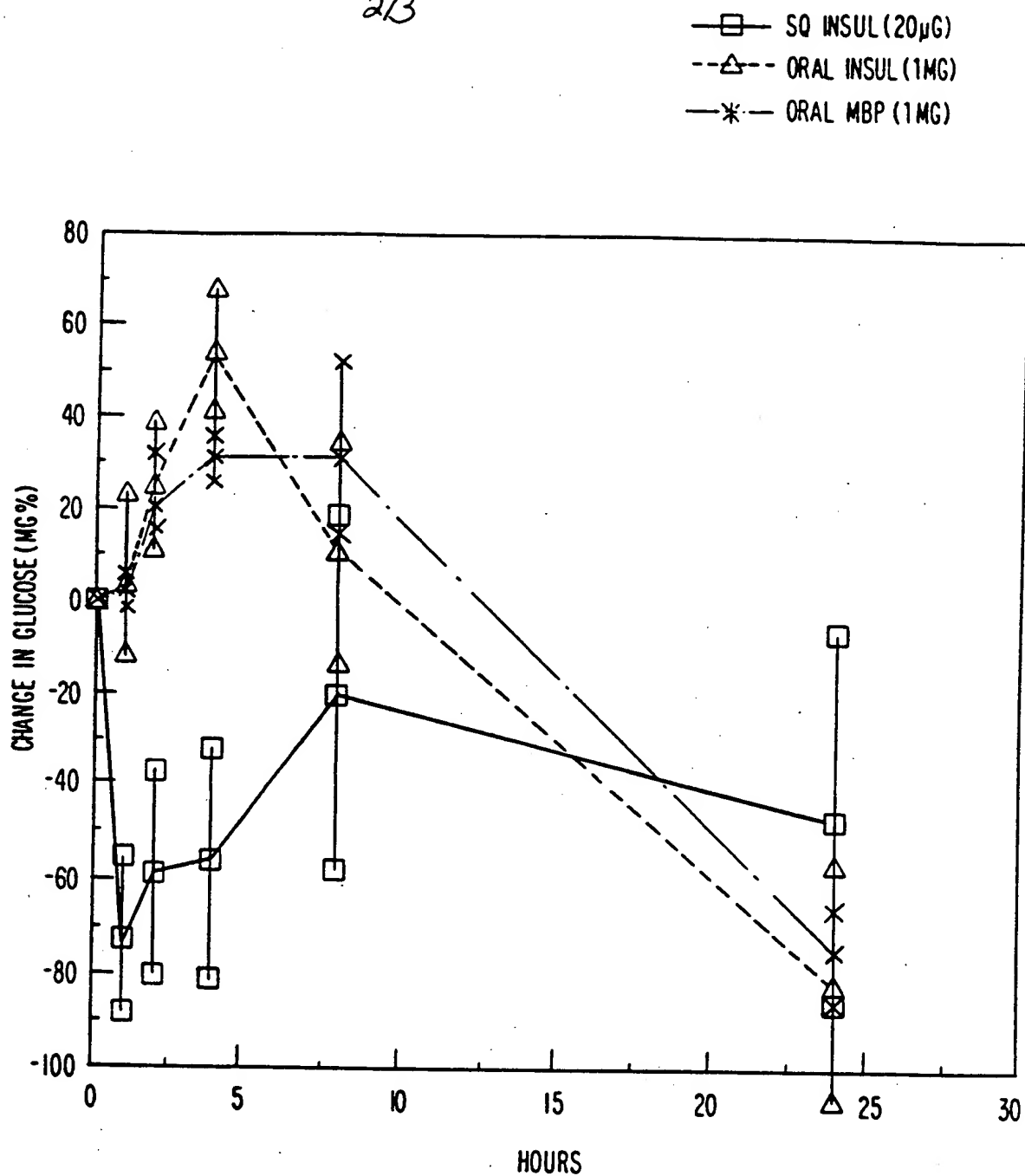


FIG. 2

SUBSTITUTE SHEET

SECRET

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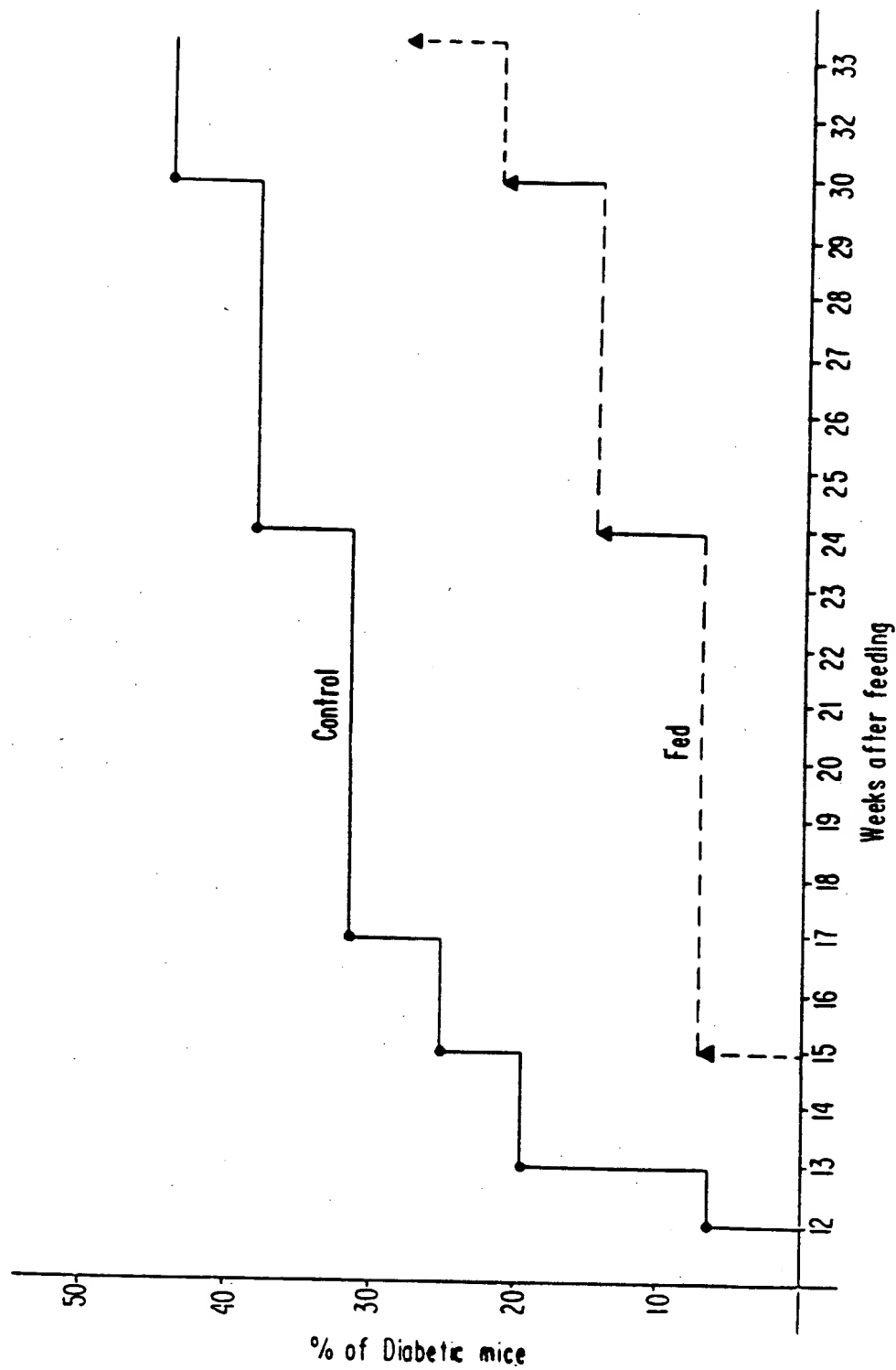


FIG. 3



# INTERNATIONAL SEARCH REPORT

International Application No. PCT/US91/07475

| <b>I. CLASSIFICATION OF SUBJECT MATTER</b> (if several classification symbols apply, indicate all) <sup>6</sup><br>According to International Patent Classification (IPC) or to both National Classification and IPC<br>IPC(5): A 61K 37/26, 9/16, 9/50<br>U.S.C1: 514/3, 4; 424/490; 435/69.4   |  |  |                       |                        |        |                             |
|--|--|--|-----------------------|------------------------|--------|-----------------------------|
| <b>II. FIELDS SEARCHED</b><br><div style="text-align: center; border-top: 1px solid black; border-bottom: 1px solid black;">Minimum Documentation Searched <sup>7</sup></div> <table style="width: 100%; border-collapse: collapse;"> <tr> <th style="width: 30%; border: 1px solid black; text-align: left;">Classification System</th> <th style="border: 1px solid black; text-align: left;">Classification Symbols</th> </tr> <tr> <td style="border: 1px solid black; padding: 5px;">US.CL.</td> <td style="border: 1px solid black; padding: 5px;">514/3, 4; 424/490; 435/69.4</td> </tr> </table> <div style="border-top: 1px solid black; padding-top: 5px;">           Documentation Searched other than Minimum Documentation<br/>           to the Extent that such Documents are Included in the Fields Searched <sup>8</sup> </div>   |  |  | Classification System | Classification Symbols | US.CL. | 514/3, 4; 424/490; 435/69.4 |
| Classification System  | Classification Symbols   |  |                       |                        |        |                             |
| US.CL.   | 514/3, 4; 424/490; 435/69.4  |  |                       |                        |        |                             |
| <b>III. DOCUMENTS CONSIDERED TO BE RELEVANT <sup>9</sup></b>   |  |  |                       |                        |        |                             |
| Category <sup>9</sup>  | Citation of Document, <sup>11</sup> with indication, where appropriate, of the relevant passages <sup>12</sup>   | Relevant to Claim No. <sup>13</sup>  |                       |                        |        |                             |
| X<br>Y   | US. A. 4,579,730. (Kidron et al.) 01 April 1986. See abstract column 1 and claims.   | 2-5,7,8,10,11,13,14<br>2-5,7,8,10,11,13,14   |                       |                        |        |                             |
| A  | The New England Journal Of Medicine, Volume 314, No. 21, Issued 22 May 1986. Eisenbarth. "Type I Diabetes Mellitus, A Chronic Autoimmune Disease". pages 1360-1368. see entire document. | 1-15   |                       |                        |        |                             |
| A,P  | Science. Volume 252. issued 05 April 1991. Marx. "Testing Of Autoimmune Therapy Begins". pages 27-28. see entire document.   | 1-15   |                       |                        |        |                             |
| A  | Diabetes Care. Volume 13, No. 7. issued July 1990. Ziegler et al. "Predicting Type I Diabetes". pages 762-775. see entire article.   | 1-15   |                       |                        |        |                             |
| <div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> <p><sup>10</sup> Special categories of cited documents: <sup>10</sup></p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="width: 45%;"> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"A" document member of the same patent family</p> </div> </div> |  |  |                       |                        |        |                             |
| <b>IV. CERTIFICATION</b>   |  |  |                       |                        |        |                             |
| Date of the Actual Completion of the International Search<br><br>05 December 1991  |  | Date of Mailing of this International Search Report<br><br><div style="font-size: 1.5em; font-weight: bold;">30 JAN 1992</div> |                       |                        |        |                             |
| International Searching Authority<br><br>ISA/US  |  | Signature of Authorized Officer<br><br>Bennett Celsa   |                       |                        |        |                             |

1. 2. 3. 4. 5. 6. 7. 8. 9. 10. 11. 12. 13. 14. 15. 16. 17. 18. 19. 20. 21. 22. 23. 24. 25. 26. 27. 28. 29. 30. 31. 32. 33. 34. 35. 36. 37. 38. 39. 40. 41. 42. 43. 44. 45. 46. 47. 48. 49. 50. 51. 52. 53. 54. 55. 56. 57. 58. 59. 60. 61. 62. 63. 64. 65. 66. 67. 68. 69. 70. 71. 72. 73. 74. 75. 76. 77. 78. 79. 80. 81. 82. 83. 84. 85. 86. 87. 88. 89. 90. 91. 92. 93. 94. 95. 96. 97. 98. 99. 100. 101. 102. 103. 104. 105. 106. 107. 108. 109. 110. 111. 112. 113. 114. 115. 116. 117. 118. 119. 120. 121. 122. 123. 124. 125. 126. 127. 128. 129. 130. 131. 132. 133. 134. 135. 136. 137. 138. 139. 140. 141. 142. 143. 144. 145. 146. 147. 148. 149. 150. 151. 152. 153. 154. 155. 156. 157. 158. 159. 160. 161. 162. 163. 164. 165. 166. 167. 168. 169. 170. 171. 172. 173. 174. 175. 176. 177. 178. 179. 180. 181. 182. 183. 184. 185. 186. 187. 188. 189. 190. 191. 192. 193. 194. 195. 196. 197. 198. 199. 200. 201. 202. 203. 204. 205. 206. 207. 208. 209. 210. 211. 212. 213. 214. 215. 216. 217. 218. 219. 220. 221. 222. 223. 224. 225. 226. 227. 228. 229. 230. 231. 232. 233. 234. 235. 236. 237. 238. 239. 240. 241. 242. 243. 244. 245. 246. 247. 248. 249. 250. 251. 252. 253. 254. 255. 256. 257. 258. 259. 260. 261. 262. 263. 264. 265. 266. 267. 268. 269. 270. 271. 272. 273. 274. 275. 276. 277. 278. 279. 280. 281. 282. 283. 284. 285. 286. 287. 288. 289. 290. 291. 292. 293. 294. 295. 296. 297. 298. 299. 300. 301. 302. 303. 304. 305. 306. 307. 308. 309. 310. 311. 312. 313. 314. 315. 316. 317. 318. 319. 320. 321. 322. 323. 324. 325. 326. 327. 328. 329. 330. 331. 332. 333. 334. 335. 336. 337. 338. 339. 340. 341. 342. 343. 344. 345. 346. 347. 348. 349. 350. 351. 352. 353. 354. 355. 356. 357. 358. 359. 360. 361. 362. 363. 364. 365. 366. 367. 368. 369. 370. 371. 372. 373. 374. 375. 376. 377. 378. 379. 380. 381. 382. 383. 384. 385. 386. 387. 388. 389. 390. 391. 392. 393. 394. 395. 396. 397. 398. 399. 400. 401. 402. 403. 404. 405. 406. 407. 408. 409. 410. 411. 412. 413. 414. 415. 416. 417. 418. 419. 420. 421. 422. 423. 424. 425. 426. 427. 428. 429. 430. 431. 432. 433. 434. 435. 436. 437. 438. 439. 440. 441. 442. 443. 444. 445. 446. 447. 448. 449. 450. 451. 452. 453. 454. 455. 456. 457. 458. 459. 460. 461. 462. 463. 464. 465. 466. 467. 468. 469. 470. 471. 472. 473. 474. 475. 476. 477. 478. 479. 480. 481. 482. 483. 484. 485. 486. 487. 488. 489. 490. 491. 492. 493. 494. 495. 496. 497. 498. 499. 500. 501. 502. 503. 504. 505. 506. 507. 508. 509. 510. 511. 512. 513. 514. 515. 516. 517. 518. 519. 520. 521. 522. 523. 524. 525. 526. 527. 528. 529. 530. 531. 532. 533. 534. 535. 536. 537. 538. 539. 540. 541. 542. 543. 544. 545. 546. 547. 548. 549. 550. 551. 552. 553. 554. 555. 556. 557. 558. 559. 560. 561. 562. 563. 564. 565. 566. 567. 568. 569. 570. 571. 572. 573. 574. 575. 576. 577. 578. 579. 580. 581. 582. 583. 584. 585. 586. 587. 588. 589. 590. 591. 592. 593. 594. 595. 596. 597. 598. 599. 600. 601. 602. 603. 604. 605. 606. 607. 608. 609. 610. 611. 612. 613. 614. 615. 616. 617. 618. 619. 620. 621. 622. 623. 624. 625. 626. 627. 628. 629. 630. 631. 632. 633. 634. 635. 636. 637. 638. 639. 640. 641. 642. 643. 644. 645. 646. 647. 648. 649. 650. 651. 652. 653. 654. 655. 656. 657. 658. 659. 660. 661. 662. 663. 664. 665. 666. 667. 668. 669. 670. 671. 672. 673. 674. 675. 676. 677. 678. 679. 680. 681. 682. 683. 684. 685. 686. 687. 688. 689. 690. 691. 692. 693. 694. 695. 696. 697. 698. 699. 700. 701. 702. 703. 704. 705. 706. 707. 708. 709. 710. 711. 712. 713. 714. 715. 716. 717. 718. 719. 720. 721. 722. 723. 724. 725. 726. 727. 728. 729. 730. 731. 732. 733. 734. 735. 736. 737. 738. 739. 740. 741. 742. 743. 744. 745. 746. 747. 748. 749. 750. 751. 752. 753. 754. 755. 756. 757. 758. 759. 760. 761. 762. 763. 764. 765. 766. 767. 768. 769. 770. 771. 772. 773. 774. 775. 776. 777. 778. 779. 780. 781. 782. 783. 784. 785. 786. 787. 788. 789. 790. 791. 792. 793. 794. 795. 796. 797. 798. 799. 800. 801. 802. 803. 804. 805. 806. 807. 808. 809. 810. 811. 812. 813. 814. 815. 816. 817. 818. 819. 820. 821. 822. 823. 824. 825. 826. 827. 828. 829. 830. 831. 832. 833. 834. 835. 836. 837. 838. 839. 840. 84